BIOLOGY.—*Reactions of the golden nematode of potatoes,* Heterodera rostochiensis *Wollenweber, to controlled temperatures and to attempted control measures.*[1] JULIUS FELDMESSER and GEORGE FASSULIOTIS, Bureau of Plant Industry, Soils, and Agricultural Engineering. (Communicated by G. Steiner.)

The golden nematode, *Heterodera rostochiensis* Wollenweber, 1923, has long been known to cause damage to potatoes in northern Europe and in the British Isles. This nematode was discovered on Long Island, N. Y., in 1941 and at the present time it appears that this is the only infected area in the United States. Since 1941 the basic biology of this potato pest and various methods of control have been under investigation.

The present study is concerned only with cysts (i.e., dead females containing viable larvae capable of infecting potato plants) and (1) differences in their resistance to control measures, (2) inhibition of the hatching of their larvae, (3) hatching of their larvae at controlled temperatures, (4) overwintering as a factor in the life-cycle of the nematode, and (5) hatching of their larvae during winter.

It has long been known that "summer cysts" of the golden nematode (i.e., cysts collected during the summer season) differed from "winter cysts" (collected during the winter season) in their resistance to control measures, such as hot-water treatment. A more careful study of these differences was occasioned in December 1949 when, in cooperation with the Division of Foreign Plant Quarantines of the U. S. Department of Agriculture, an attempt was made to find suitable treatment for frozen shipments of lily-of-the-valley pips containing cysts of *H. rostochiensis*, being imported from the West Zone of Germany. Full results of these investigations will be published elsewhere. The present study, however, concerns summer and winter cysts taken from the soil of Long Island, N. Y., put through treating schedules to determine whether or not they differed in reactions to treatments.

Prior to 1947, B. G. Chitwood, in work conducted on Long Island, had washed cysts from soil for winter use during the previous summers. These cysts were maintained at room temperature until they were used in experiments during the winter. The same procedure was followed by the senior author of the present study during the winters 1947–1948 and 1949–1950 with one modification: the cysts were maintained at 70°F. in a constant-temperature chamber until the time they were used. Therefore when the treating schedule was started in December 1949, a supply of summer cysts was on hand. To secure the winter cysts, infected soil was removed from a field at Hicksville during the week of December 5, 1949, and allowed to thaw out in an unheated shed. The cysts were washed out of the soil through a U.S. Standard no. 60 sieve out-of-doors and transferred immediately to the laboratory refrigerator set at 40°F. Cysts of both categories were removed from their respective chambers immediately prior to being treated.

Cysts of each type, summer and winter, in one gram lots contained in small nylon bags, were exposed to hot water in a thermoregulator-equipped hot water bath, and to methyl bromide in a fumigation chamber. The bags exposed to hot water were pre-wet to insure the escape of air from them and were immersed in water at room temperature immediately after the exposure periods. After treatment, each lot of cysts was divided into two parts and examined for viability at periods varying from three to four weeks later. One half of each lot was examined under a binocular dissecting microscope and the numbers of viable and non-viable cysts counted, the presence of one or more viable larvae in any cyst indicating that the cyst was viable. The second half was exposed to the stimulatory effect of potato root leachings (O'Brien and Prentice, 1931) and checked twice weekly for evidences of larval hatching.

The two categories of cysts showed different reactions to control measures, the winter cysts exhibiting a greater resistance to various types of control measures than the summer cysts. Triffitt and Hurst (1935) state that exposures to water at 116°F. for

[1] Report of a study made under the Research and Marketing Act of 1946. Received September 13, 1950.

45 minutes, 118°F. for 30 minutes and 130°F. for 5 minutes are lethal to the contents of the cysts of *H. rostochiensis*. In the present study, however, 116°F. for 45 minutes is a borderline treatment: two replications contained viable cysts of both categories after a two week exposure to leachings and two replications contained no viable cysts. The winter cysts show greater resistance to methyl bromide fumigation also, based on a thirty percent viability in the controls. An average of 23.8 percent of the winter cysts remained viable after exposures of four hours to four pounds per one thousand cubic feet of methyl bromide at normal atmospheric pressure and at 15 and 27 inches of vacuum. This represents a 20.7 percent kill of the formerly viable cysts. On the other hand, an average of 2.6 percent of the summer cysts remained viable when exposed to the same treatments, the kill, when compared to the control, being 91.3 percent.

This wide divergence in efficacy suggests one of the possible reasons why soil fumigation for the control of cyst-forming nematodes is more effective when applied immediately after a late summer or early autumn harvest, rather than in the spring before planting. Nematode cysts are in the summer condition late in summer and early in autumn and are more susceptible to soil treatments at that time. It is suggested that the proportionate increase of bound water within the larval protoplasm (at the expense of the free water), a device that Payne (1927a, 1927b, 1928), Robinson (1928), and Saccharov (1930) have shown to be a factor in the survival of insects at low temperatures, may be involved here. *Synchroa punctata* Newman and *Popillia japonica* Newman have been shown to be less susceptible to both extremes of temperature when most of their free water has been lost.

## INHIBITION OF LARVAL HATCHING

Since golden-nematode cysts varied in their reactions to control measures, it was decided to investigate the effects, if any, of these treatments on the ability of larvae to hatch out of cysts. A search of the literature showed instances of inhibition of the hatching reaction of larvae of *H. rostochiensis* contained within the cysts. Carroll (1933) showed that exposure of cysts for two weeks to 1:100 and 1:250 parts of urea afterward resulted in complete inhibition of hatching. A 2-week delay resulted from exposure to 1:1000 parts. Hurst and Triffitt (1935) and Carroll and MacMahon (1937) found that ferric oxide added to potato root leachings delayed hatching. O'Brien and Gemmell, Prentice, Wylie (1939) state that a calcium chloroacetate treatment afterwards caused considerable delay in hatching. Smedley (1939) found that exposure of samples of one hundred cysts each to fumes of phenyl, ethyl or n-butyl isothiocyanate for 24 hours was sufficient to prevent hatching in potato root leachings afterwards for a period of two months; that exposure of samples to a 0.004 percent solution of ethyl isothiocyanate inhibited hatching for six weeks; and that the exposure of samples to 0.004 percent and 0.001-percent solutions of n-butyl isothiocyanate resulted in 24 larvae hatching after the 0.004 percent and 300 after the 0.001 percent. In the untreated control 8,500 larvae hatched out.

Cysts of both categories, winter and summer, used to check the efficacy of 4 pounds of methyl bromide per 1,000 cubic feet for four hours as a means of control were used in the present investigation. Cysts were treated on three separate occasions, and altogether seven treated lots were checked. Examinations for indications of viability were made in two ways: visually, under the binocular dissecting microscope, and by examining dishes containing treated cysts and potato-root leachings for the presence of hatched-out larvae.

With the aid of the binocular dissecting microscope, viable larvae were judged to be present in cysts of all of the treated lots. Untreated controls contained hatched-out larvae after 24 hours. Dishes containing cysts from three of the lots contained hatched-out larvae after a 1- to 2-week exposure to the stimulatory effect of potato-root leachings. Dishes containing cysts from the other four lots, however, did not contain hatched-out larvae in the same period. These cysts were then cut open in the leachings and viable larvae were found. These larvae exhibited motility when stroked with the shaft of a fine needle in the region of the circum-esophageal nerve ring. No explanation for these differing responses to potato-root leachings can be made at this time.

The results obtained with nematode cysts treated with four pounds of methyl bromide per 1,000 cubic feet for four hours indicate the existence of a period of inhibition of from one to two weeks after exposure to leachings. However, these cysts were exposed to leachings at periods varying from three to four weeks after treatment. Therefore, the total period of post-treatment inhibition may be longer when cysts are exposed to potato root leachings immediately after treatment.

It is possible that a revision of screening techniques for nematocides is in order. The absence of hatched-out larvae in treated material exposed to leachings at periods long enough to allow hatching in the controls cannot be considered as a definite indication of killing properties.

## HATCHING AT CONTROLLED TEMPERATURES

There are several indications in the literature of the interrelations of temperature and the ability of *Heterodera* sp. larvae to hatch from cysts. Baunacke (1922) established two low temperature limits for the escape of larvae from cysts of *Heterodera schachtii* Schmidt: 10°C. for the escape of those that have hatched within the cyst and 18°C. for larvae that have to free themselves from their egg cases before they can escape from the cyst. These latter, according to the author, are not capable of movement as energetic as the former. Triffitt (1930), working with *H. rostochiensis* cysts that had been air-dried and stored in an unheated shed, concluded that a period of dormancy exists in the winter months during which time the larvae "will not hatch even under optimum conditions of temperature and moisture and in the proximity of potato root excretions." Miles (1930), in a study of "potato sickness" under field conditions associated intensity of root invasion with soil temperatures of 59°F. to 60°F. Since two categories of *H. rostochiensis* cysts, winter cysts and summer cysts, exist as far as resistance to hot water and methyl bromide treatments are concerned and since both categories show hatching responses to potato root leachings, it was decided to expose both categories to varying temperatures in leachings and in tap water to determine differences, if any, in hatching responses.

One gram lots of cysts, each containing approximately 300 viable cysts, were immersed in potato root leachings and in tap water in petri dishes at the following temperatures: 40°F., 50°F., 70°F., 80°F., and 100°F. The dishes were maintained at these temperatures for 16 hours in constant temperature chambers. At the end of the 16-hour period, the hatched larvae were separated from the cysts through a U.S. Standard no. 100 sieve and counted under the binocular dissecting microscope. The results are shown in Table 1.

The average numbers of hatched larvae from summer cysts show a significantly higher response, both to tap water and to potato-root leachings, at all temperatures.

Table 1.—Number of Heterodera rostochiensis Larvae Hatching at Controlled Temperatures during a 16-Hour Period in Tap Water and in Potato Root Leachings

| Rep. No. | Summer cysts | | Winter cysts | |
|---|---|---|---|---|
| | Tap water | Leachings | Tap water | Leachings |
| **40°F** | | | | |
| A | 32 | 31 | 4 | 5 |
| B | 55 | 25 | 3 | 9 |
| C | 38 | 20 | 4 | 7 |
| Aver. | 41.6 | 25.3 | 3.8 | 7.0 |
| **50°F** | | | | |
| A | 53 | 41 | 6 | 1 |
| B | 47 | 30 | 20 | 3 |
| C | 33 | 54 | 8 | 3 |
| Aver. | 44.3 | 41.7 | 11.3 | 2.3 |
| **70°F** | | | | |
| A | 109 | 205 | 4 | 7 |
| B | 113 | 188 | 2 | 4 |
| C | 110 | 201 | 1 | 6 |
| Aver. | 110.7 | 198.3 | 2.3 | 5.7 |
| **80°F** | | | | |
| A | 53 | 131 | 3 | 9 |
| B | 60 | 88 | 1 | 14 |
| C | 73 | 95 | 4 | 9 |
| Aver. | 62.0 | 104.7 | 2.7 | 10.7 |
| **100°F** | | | | |
| A | 54 | 40 | 3 | 13 |
| B | 40 | 47 | 0 | 2 |
| C | 44 | 44 | 3 | 3 |
| Aver. | 46.0 | 43.7 | 2.0 | 6.0 |

The stimulatory effects of tap water seem to be as marked as those of the potato root leachings at 40°F., 50°F., and 100°F. At the temperatures more commonly associated with the height of the growing season, however, potato root leachings do show a greater effect. The averages of the winter cysts show uniformly lower hatching responses at all of the temperatures both in potato-root leachings and in tap water.

A search of the literature dealing with dormancy in the invertebrates indicates that experimental work, thus far has been restricted largely to the insects. Payne (1927a, 1927b, 1928) found that survival of *Synchroa punctata* Newman at freezing temperatures is associated with the organism's ability to undergo dehydration. She specifies that the dehydration involves the loss of free water only. Cold hardiness was produced by experimental dehydration of individuals to half their body weights. Such individuals survived temperatures as low as −28°C. Robinson (1926) points out that low temperatures are believed to cause the streaming of free water from the cells to the intercellular spaces. Formation of ice crystals in the intercellular spaces causes death mechanically. Cold hardy species, on the other hand, can bind their water into hydrophilic colloids and freezing does not occur at these temperatures. Saccharov (1930) states that cold hardiness of insects depends on the presence of a minimum quantity of water and the ability of the organism to accumulate fat. All hibernating stages of insects prepare themselves to withstand cold by reducing the quantity of free, easily freezable water in their tissues. These facts suggest several of the processes possibly involved in the adaptation of *Heterodera rostochiensis* to temperature extremes. Since both categories of cysts can exist at the same time, it is assumed that the winter condition is not an inherent periodic manifestation in the life-cycle. The winter condition; rather, is due to the adaptation of the larvae to lower temperatures. Another possibility is that the winter condition (i.e., dormancy) does exist as an integral part of the life-cycle, its degree, however, being influenced greatly by temperature. It is not yet known how long an exposure to a significantly higher or lower temperature is necessary for the nematode to readjust from one type to the other.

The data on the invasion of potato roots by *H. rostochiensis* larvae during the less active period and the data on the hatching of larvae between 40°F. and 100°F. are being augmented by work now in progress to determine the actual limits of activity of the nematode.

## OVERWINTERING AS A FACTOR IN THE LIFE-CYCLE

An experiment was set up to determine whether or not overwintering, i.e., exposure to Long Island, N.Y., winter soil temperatures of 30°F–50°F, might play a role in the activation or maturing of larvae within cysts of *Heterodera rostochiensis.*

Pots containing potato seed pieces and sterile nematode-free soil were inoculated with large uncounted numbers of viable golden nematode larvae on September 16, 1949. The pots were kept in the greenhouse until December 1949. After the tops of the plants had died back, the pots were brought into the laboratory. At no time were the pots exposed to temperatures lower than 55°F. During April 1950, the nematode cysts, all of which had developed during the period between September and December 1949, were washed from the soil. Some were exposed to potato root leachings and others were placed in contact with potato seed pieces in nematode-free soil.

Subsequently, the dishes of cysts and leachings contained hatched larvae within two days and the seed pieces in infected soil bore white nematode cysts in early May 1950, indicating that soil temperatures between 30°F and 50°F under Long Island, N.Y. conditions play no part in the activation or maturing of larvae of *H. rostochiensis.*

## HATCHING DURING WINTER

The life-cycle of *Heterodera rostochiensis* has been studied in detail by Chitwood and Buhrer (1946). They state that the course of the individual life-cycle, the period from embryonated eggs to embryonated eggs, may be not less than 38 days and does not exceed 48 days. In this study, Chitwood and Buhrer's observations were compared with the course of the life-cycle of the nematode during the winter. Their findings are confirmed in the present study by the results obtained with the use of winter cysts.

Soil containing golden nematode cysts was dug from the laboratory's infested field on January 8, 1950. At that time, the top three inch layer of soil was frozen and the temperature of the underlying soil was 33°F to 34°F. The soil was allowed to thaw out overnight in an unheated shed and the cysts were washed out on January 10, 1950. The cysts were then combed through sterile nematode-free soil. Potato seed pieces of the Katahdin variety in which dormancy had been broken were planted in six inch pots in this soil on the same day. The pots were kept in benches in the greenhouse where temperatures fluctuated between 50°F and 65°F during the growing period.

On March 10, 1950, white cysts were observed at the exterior of the root balls. On March 23, 1950, 72 days after the planting date, the cysts were of a deep yellow color and contained embryonated eggs (Fig. 1). Four to five weeks may be allowed for the appearance of potato roots at the exterior of a 6-inch soil ball. Therefore, larvae from cysts near the exterior of the soil ball entered

Fig. 1.—Root ball of Katahdin potato plant with cysts of the golden nematode attached. Pictures taken March 23, 1950.

external roots growing out toward them in the four to five weeks after the planting on January 10. Yellow cysts containing embryonated eggs formed during a period of 37 to 44 days after the 4- to 5-week period.

In addition to the qualitative work mentioned above, a quantitative study showing that the nematode can reproduce at normal rates during the winter was made by the senior author of the present study (1949). Potato-seed pieces were exposed to known quantities of cysts in nematode-free soil in pots. The pots were kept in eight constant-temperature tanks in which temperatures were maintained by thermo-regulators. Each tank held eight pots containing cyst inocula of 1, 5, 16, 32, 450, 9000, 22,500, and 45,000 viable cysts, respectively. The rates of increase of new generation cysts resulting were inversely proportional to the quantity of inocula, the average rate of increase in pots with one viable cyst being 45.0 times the inoculum and the averge for the 45,000 cysts being 1.74 times the inoculum. The average rates of increase by tanks were as high as 13.6, with five of the tanks showing a rate of increase of 10.5 or better.

These observations serve as an indication that golden nematode cysts in the winter condition (from soil at freezing and near freezing temperatures) will infect potato roots when kept at temperatures approximating those of the normal growing season.

## SUMMARY

Experiments were conducted which indicate that:

1. Cysts of the golden nematode, *Heterodera rostochiensis*, fall into two categories as far as susceptibility to methyl bromide fumigation and hot water treatments are concerned: the contents of winter cysts maintained at approximately 40°F. being less susceptible to control measures than those summer cysts maintained at 70°F.

2. Exposure of larvae within cysts to sublethal control measures may afterward cause an inhibition of the hatching response in potato-root leachings.

3. Golden nematode larvae will hatch out at temperatures as low as 40°F. when exposed to potato-root leachings and to tap water for 16 hours.

4. Larvae of 1-year-old cysts do not have to overwinter under Long Island, N.Y., field conditions in order to hatch out.

5. Larvae of cysts removed from field conditions in January 1950, when soil temperatures were at the freezing level, hatched out and infected potato roots during the same month under greenhouse conditions.

6. Hatching of golden-nematode larvae occurs under controlled temperatures during the winter months. Rates of reproduction compare favorably with those of natural infections during the summer.

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ENTOMOLOGY.—*Notes on Trichopodini (Diptera, Larvaevoridae), with description of a new parasite of cotton stainers in Puerto Rico.*[1] CURTIS W. SABROSKY, Bureau of Entomology and Plant Quarantine.

In Puerto Rico attempts were made in 1941 and 1942 to introduce from Peru a larvaevorid fly, *Acaulona peruviana* Townsend, as a parasite of cotton stainers, *Dysdercus* spp. Subsequent collections to determine whether that species had become established revealed the presence of an undescribed parasite, apparently a native species. In the taxonomic study of this new species, the New World genera of the tribe Trichopodini (*sensu* Townsend) were reviewed, and although a complete revision is not possible at this time, some results may be of aid to other workers in the future.

For convenience, the present paper is confined to the Trichopodini in the sense of Townsend. Certainly some genera of Townsend's Phasiini, however, are much closer to Trichopodini than to other phasiine genera, especially in the type of male and female genitalia (e.g., *Ectophasiops* Townsend). A similar distinction has been pointed out by Brooks and by Dupuis in recognizing two groups of genera within the Phasiini, based on reproductive habit and type of female genitalia. A natural grouping would seem to call for considerable rearrangement, with combination of parts or all of several tribes.

In almost all proposed genera of the Trichopodini either the fundamental structure is so similar or the characters so overlap that one must perforce either synonymize extensively or, with Townsend, construct unsatisfactory keys on variable and ineffective characters. One feature that seems not to have been used previously is the chaetotaxy of the scutellum, and this is so markedly different in two groups that it is a significant and highly useful initial division. In some cases, also, it is possible that differences in the form of the genitalia may justify generic recognition.

Complete citations of genotypes have been omitted for purposes of this contribution. They are given in full in Townsend (*Manual of myiology*, pt. 7: 9–35. 1938).

[1] Received September 15, 1950.

### KEY TO THE NEW WORLD GENERA OF TRICHOPODINI

*Key to Groups*

1. Scutellum with basal scutellar bristles much longer than apicals, widely divergent, and inserted midway between basal corner of scutellum and apical bristles (Fig. 8); apical cell long petiolate. *Xanthomelanodes* complex
   Scutellum with basal bristles not obviously longer than apicals, directed posteriorly or curving mesad, and inserted quite near basal corner of scutellum (Fig. 9); apical cell open, closed at margin, or very short petiolate . . 2
2. Posterior tibia ciliate dorsally with a row of close-set, flattened bristles, which may or may not be obviously longer than diameter of tibia . . . . . . . . . . . . . . . . *Trichopoda* complex
   Posterior tibia entirely without flattened bristles . . . . . . . . . . . . . . . . . . *Acaulona* complex

*Xanthomelanodes Complex*

1. Second segment of arista elongated, 3 times length of basal segment and 5 times it own breadth; third antennal segment elongated, 3 times length of second segment. *Xenophasia* Townsend
   First 2 segments of arista short; third antennal segment not elongated. *Xanthomelanodes* Townsend

*Trichopoda Complex*

1. Postcoxal area, between hind coxae and first abdominal sternite, membranous and characteristically sunken or shriveled; front somewhat narrowed at vertex, the sides parallel on basal half, then suddenly diverging (Fig. 4) . . . . . . . . . . . . . . . . . . 2
   Postcoxal area sclerotized, the lateral plates not distinctly separated by a sunken, membranous or weakened area; front not as